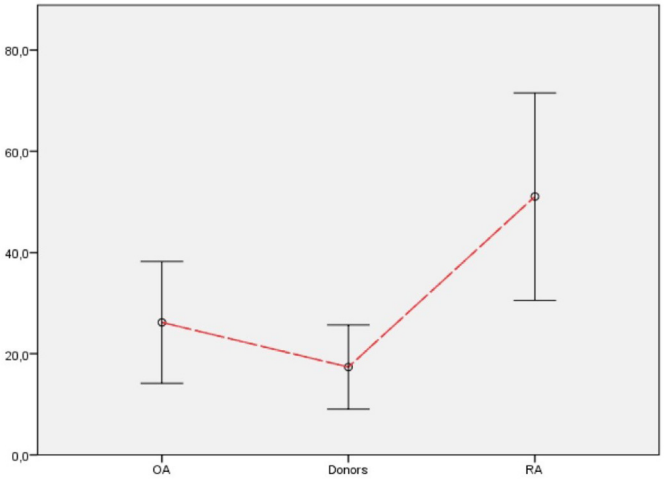
role of T cells, primarily Th17 cells, are now well recognised. On the other hand data about immunological profile in OA are limited, because OA has long been regarded in the past as a no inflammatory disease. Our study aim to measure the distribution and the activation degree of CD4+ Th17 in peripheral blood of OA, RA and age-matched healthy controls.
Methods: Patients with established diagnosis of RA according to ACR/EULAR 2010 criteria, knee or hip OA according to ACR criteria and volunteers healthy blood donors were eligible. Other inclusion criteria were a DAS28 between 3.2 and 5.1 or a WOMAC Likert score more than 50, respectively. Exclusion criteria were the presence of other autoimmune diseases, tumours or secondary osteoarthritis. Finally no changes in rheumatologic drugs were allowed from 3 months before enrolment. Multichannel flow cytometry was used for T cells subpopulation distinguishing and quantification using monoclonal antibodies against CD3, CD4, CD8, CCR6, CD38, CXCR3 and HLA DR. Participants were informed about the aim of the project and gave their written consent. The project was accepted by the Local Ethic Committee.
Results: We analysed blood samples of 91 subjects (75 females, 16 males). 15 Patients with well-defined RA, 56 with hip or knee OA and 20 healthy controls. Mean age was 45 ± 2.7 years old ($p > 0.05$ between groups). Blood samples from the RA patients had significantly higher count of CD4+CD38+DR+ (activated CD4 T cells) and Th17 (CCR6+CXCR3-) cells as compared to OA patients and control group (Figure) ($P < 0.01$). Furthermore the samples from the OA patients had an higher percentage of activated CD4 T cells and Th17 cells as compared to control group ($P < 0.05$)
Conclusion: According to the latest view of OA disease pathogenesis, our preliminary results give support to the hypothesis that OA may also (like RA) be a disease with an immunological/inflammatory involvement. In fact it seems that there is a quantitative but non qualitative difference in Th17 cells profile, including the expression of activation markers, between RA and OA.



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PERIODONTITIS DISEASE AS A MODEL OF INFLAMMATION AND BONE REMODELLING TO STUDY OSTEOARTHRITIS DRUG EFFECTS. RESULTS FROM A PILOT STUDY IN OA PATIENTS TREATED WITH CHONDROITIN SULFATE

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Purpose: Periodontitis is an inflammatory disease that affects 70% of the adult population. Its most important consequence is the loss of bone supporting the teeth. Its medical treatment so far has not shown to be effective so only different surgical techniques are useful in advanced stages. Osteoarthritis (OA) and periodontitis share physiopathological characteristics. OA patients also suffer from an important inflammatory component in the soft tissue and bone structural modifications. Because of the evolution of the disease, development of new treatments is difficult. Periodontitis could be a model for the study of OA, where the changes of tissues could be observed in a more directly way. Chondroitin sulfate (CS) has shown a positive effect in different chronic inflammatory diseases such as psoriasis, inflammatory bowel disease and OA. Periodontitis is the most prevalent inflammatory disease, but currently without specific treatment. New approaches for a safety and effective pharmacological treatments are required. The purpose of this study was to demonstrate that an effective drug in OA pathology may improve both the inflammatory symptoms and the bone resorption occurred in periodontal disease. This improvement would be reflected in changes in biochemical markers of inflammation and bone metabolism.
Methods: Observational, prospective, pilot study in 26 patients diagnosed with knee OA and periodontitis. Patients were treated with CS 800 mg/day(Condrosan®, Bioibérica SA) for 12 months. The Löe and Silness gingival index (used to assess soft tissue damage) and CPITN (Community Periodontal Index of Treatment Needs) index were evaluated at 0, 3, 6, 9 and 12 months with saliva collection. Orthopantomographys were performed at 0, 6 and 12 months to evaluate bone damage. Vertical lesions were measured using a periodontal probe at 0, 6 and 12 months. Different concentration of inflammatory markers (TNF -α, IL -1β, IL-18 and PGE_2) and bone metabolism (OPG, OPN, RANKL and MMP-8) were quantified in the saliva by ELISA or protein array. During the study, patients were asked to continue their usual oral hygiene without additional treatments. Statistical analysis was performed using Wilcoxon test for paired samples.
Results: Löe and Silness index decreased significantly after 6, 9 and 12 months of treatment ($p = 0.004$, 0.007 and 0.002 respectively). In contrast, no significant changes were observed in CPTIN values, probably due to the fact that only the most affected tooth is studied and it can differ between visits. Both orthopantomography as well as the vertical lesions evolution, showed significant improvement at 12 months ($p = 0.009$) and 6 and 12 months respectively ($p = 0.002$ and $p = 0.016$). For biochemical analysis results, patients with no gingival improvement were used as control group. The results show a better performance in the group of responders to treatment for inflammatory markers PGE_2, TNF-α and IL-1β , as well as bone metabolism markers, OPN, MMP -8, and the ratio OPG/RANKL. Responders groups PGE_2 levels showed a significant decreased after 12 months of treatment ($p = 0.033$), whereas IL-1β levels in non responding patients suffered a significant increased after 3 and 6 months of treatment ($p = 0.017$)
Conclusions: CS improves soft tissue inflammation after 6 months of treatment and bone support at 12 months in periodontal disease, similar to the already known effect of the drug in OA disease. This improvement is reflected in markers of inflammation and bone metabolism in saliva. Due to its efficacy and safety profile, CS is postulated as a good candidate for the treatment of this disease.

Intervertebral disc

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ADIPOKINES AND THE INTERVERTEBRAL DISC: DOES A BIOCHEMICAL LINK EXIST BETWEEN OBESITY AND BACK PAIN?

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Purpose: Obesity is a significant risk factor for development of low back pain and intervertebral disc (IVD) degeneration. The mechanism underlying this link is unclear but is commonly thought to arise from altered loading. However, adipokines such as leptin and adiponectin, produced by adipose tissue, are now known to be involved in degradative processes particularly in articular joints. We propose a similar link exists between these adipokines and intervertebral disc degeneration. Obese individuals are known to have higher concentrations of serum adipokines, there is increased local fat in chronic spinal conditions potentiating an increase in local adipokine levels and disc cells are reported to have leptin receptors and can synthesise leptin.
The aim of the study was to identify responses of nucleus pulposus (NP) and outer annulus fibrosus (OA) cells to leptin and adiponectin. Once identified, we proceeded to determine if synergistic effects exist in the presence of other pro-inflammatory cytokines.
Methods: Bovine intervertebral discs were used as a model system. Freshly isolated NP and OA cells embedded in 3D alginate beads, were

cultured under varying concentrations of leptin alone, adiponectin alone or together with the pro-inflammatory cytokines TNF-α and IL-1β. Lactate was used to assess energy metabolism. Active and proMMP-2 and -9 in the culture medium were measured using gelatin zymography. Western blotting was used to assess levels of MMP-1, -3 and -13 and quantitative real-time PCR was used to assess expression levels of anabolic and catabolic genes.

Results: Leptin influenced cellular metabolism of both the NP and OA cells. Furthermore leptin led to decreased glycosaminoglycan production and significantly increased production of MMP-2, -3 and -9, by both cell types at the protein level. Similar results were seen with adiponectin. Gene expression of the matrix genes aggrecan, collagen I and collagen II fell with increasing concentrations of leptin. Most importantly, in the presence of leptin, the expression of both TNF-α and IL-1β were significantly upregulated. Addition of leptin to medium containing the pro-inflammatory cytokines, demonstrated a marked synergistic effect on energy metabolism and the production of certain proteases, especially MMP-2.

Conclusions: Our results show that leptin can upregulate proteases involved in degenerative processes in the IVD, and that this effect is potentiated in the presence of pro-inflammatory cytokines such as TNF-α and IL-1β. Leptin levels are increased markedly in obese patients and hence a biochemical mechanism may be involved in the association between obesity, disc degeneration and back pain particularly in an inflammatory environment

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OSTEOGENIC DIFFERENTIATION OF IVD CELLS: INDUCTION OF BIOLOGICAL FUSION.

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Purpose: Much focus has been placed on the development of methods for biological repair or regeneration of the degenerate intervertebral disc (IVD), with varying success in humans and animal models. The current 'gold standard' treatment for IVD degeneration is fusion of the vertebral space, requiring invasive and painful surgery with extensive metalwork. Unlike IVD regeneration, little emphasis has been placed on the development of biological fusion. We have investigated the ability for IVD cells to differentiate osteogenically and aim to determine the 'best' method for bone formation. Ideally, this will result in the development of a method to induce the degenerate (and painful) IVD to turn itself to bone and fuse the vertebral space and thus remove the need for major surgery for the patient.

Methods: Monolayer cultures of IVD cells and mesenchymal stromal cells (MSCs) isolated from samples obtained from patients undergoing routine surgery for IVD disorders were treated with standard culture media, osteogenic media (including 100 nM dexamethasone, 10 nM β-glycerophosphate and 50 μM L-ascorbic acid-2-phosphate) or 1,25 dihydroxyvitamin D3 (VitD3) at 0.1, 1 or 10 nM for 21 days. Treated cells were histochemically stained for alkaline phosphatase activity, a marker of osteogenic differentiation.

Results: VitD3 produced a dose-related increased production of alkaline phosphatase by both MSCs and IVD cells, compared to standard culture media. However, the response to osteogenic media was greater. Generally, the response of IVD cells under all culture conditions was less than seen with MSCs, although the trends were always the same.

Conclusions: These preliminary results show that VitD3 can induce osteogenic differentiation of IVD cells, but often to a lesser extent than media containing dexamethasone, β-glycerophosphate and L-ascorbic acid-2-phosphate or that seen from MSCs. This is encouraging in the search for a method to induce biological fusion of the vertebral space, but further work using other osteogenic factors is required.

Joint Morphology and/or Morphometry

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STUDY OF CARTILAGE DAMAGE INDEX WITH JOINT SPACE NARROWING AND KELLGREN-LAWRENCE GRADE

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Purpose: While cartilage morphometry on magnetic resonance (MR) imaging is increasingly accepted as an outcome measure for clinical trials among individuals with osteoarthritis (OA), it remains burdensome, which limits its utility in large studies. We recently developed a rapid knee cartilage quantification method that has been validated with joint space width, joint space narrowing, static alignment. As another step in our validation process we wanted to confirm the cartilage damage index (CDI) can also detect a previously described association between cartilage damage and Kellgren-Lawrence (KL) grade with the exception of KL = 2, which has cartilage thickening.

Methods: We selected 102 participants from the Osteoarthritis Initiative (OAI) who had a diverse range of JSN (0 to 3) and KL grades (0 to 4) and for whom 3D double-echo steady-state sagittal images were available. MRIs were obtained on four 3-Tesla systems (0.37 mm × 0.37 mm, 0.7 mm slice thickness). One reader used customized software to measure the CDI in the medial femur and tibia from the baseline and 24-month visit (ICCs3,1 = 0.95 to 0.99). Central readers determined semi-quantitative assessments of radiographic knee OA severity (KL grade and modified OARSI-atlas based medial JSN scores) using the weight-bearing posterior-anterior fixed-flexion knee radiographs from the baseline OAI visit (radiographic readings are publicly available at http://oai.epi-ucsf.org/; kxr_sq_bu_00 [version 0.5]). To account for different skeletal

Cartilage damage index stratified by baseline medial JSN grade

Cartilage Measure	JSN = 0(n = 43) mean (SD)	JSN = 1(n = 25) mean (SD)	JSN = 2(n = 26) mean (SD)	JSN = 3(n = 3) mean (SD)	p-value for Trend
Femur CDI (Baseline)	747.95 (143.69)	664.55 (98.89)	521.57 (108.73)	308.95 (171.85)	<0.01
Femur CDI (Baseline)	389.56 (78.60)	368.12 (59.16)	292.77 (71.57)	149.53 (113.88)	<0.01
Tibiofemoral CDI (Baseline)	1137.50 (203.22)	1032.70 (149.94)	814.34 (152.48)	458.48 (278.78)	<0.01
Femur CDI (Change)	−2.95 (48.95)	−19.33 (57.29)	−47.15 (60.92)	−99.30 (90.47)	<0.01
Tibia CDI (Change)	−9.31 (26.30)	−26.25 (26.41)	−45.00 (41.63)	−43.86 (44.65)	<0.01
Tibiofemoral CDI (Change)	−12.26 (63.30)	−45.58 (71.29)	−92.15 (82.51)	−143.2 (134.52)	<0.01

Cartilage damage index stratified by baseline KL grade

Cartilage Measure	KL = 0 (n = 4) mean (SD)	KL = 1 (n = 19) Mean (SD)	KL = 2 (n = 40) mean (SD)	KL = 3 (n = 34) mean (SD)	KL = 4 (n = 5) mean(SD)	p-value for Trend
Femur CDI (Baseline)	676.99 (90.01)	657.45 (151.46)	712.84 (117.43)	599.35 (169.07)	527.67 (332.25)	0.02
Tibia CDI (Baseline)	416.01 (97.53)	350.20 (58.16)	378.33 (67.55)	330.49 (94.90)	248.59 (169.46)	<0.01
Tibiofemoral CDI (Baseline)	1093.0 (179.14)	1007.7 (192.65)	1091.2 (170.23)	929.83 (248.63)	776.26 (497.49)	0.01
Femur CDI (Change)	−38.28 (57.02)	−15.08 (52.16)	−4.95 (52.98)	−40.33 (58.57)	−38.70 (107.60)	0.15
Tibia CDI (Change)	−43.46 (50.24)	−18.96 (27.01)	−14.33 (27.37)	−36.19 (38.95)	−36.19 (38.95)	0.23
Tibiofemoral CDI (Change)	−81.74 (106.83)	−34.04 (58.88)	−19.29 (70.13)	−76.52 (79.70)	−65.35 (143.93)	0.12